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Conspecific disturbance contributes to altered hibernation patterns in bats with white-nose syndrome

James M. Turner [a,1], Lisa Warnecke [a,1], Alana Wilcox [a], Dylan Baloun [a], Trent K. Bollinger [b], Vikram Misra [c], Craig K.R. Willis [a,*]

[a] Department of Biology and Centre for Forest Interdisciplinary Research, University of Winnipeg, Winnipeg, MB R3B2E9, Canada
[b] Department of Veterinary Pathology and Canadian Wildlife Health Cooperative, University of Saskatchewan, Saskatoon, SK S7N5B4, Canada
[c] Department of Veterinary Microbiology, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK S7N5B4, Canada

HIGHLIGHTS

- Bats infected with *Pseudogymnoascus destructans* synchronised arousals from torpor.
- Bats often aroused sequentially, but normothermic phases did not overlap entirely.
- Rewarming rates did not differ between infected and control bats.
- Rewarming rate was not affected by clustering behaviour.
- We suggest disturbance by aroused, infected bats affects hibernation patterns.

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ABSTRACT

The emerging wildlife disease white-nose syndrome (WNS) affects both physiology and behaviour of hibernating bats. Infection with the fungal pathogen *Pseudogymnoascus destructans* (*Pd*), the first pathogen known to target torpid animals, causes an increase in arousal frequency during hibernation, and therefore premature depletion of energy stores. Infected bats also show a dramatic decrease in clustering behaviour over the winter. To investigate the interaction between disease progression and torpor expression we quantified physiological (i.e., timing of arousal, rewarming rate) and behavioural (i.e., arousal synchronisation, clustering) aspects of rewarming events over four months in little brown bats (*Myotis lucifugus*) experimentally inoculated with *Pd*. We tested two competing hypotheses: 1) Bats adjust arousal physiology adaptively to help compensate for an increase in energetically expensive arousals. This hypothesis predicts that infected bats should increase synchronisation of arousals with colony mates to benefit from social thermoregulation and/or that solitary bats will exhibit faster rewarming rates than clustered individuals because rewarming costs fall as rewarming rate increases. 2) As for the increase in arousal frequency, changes in arousal physiology and clustering behaviour are maladaptive consequences of infection. This hypothesis predicts no effect of infection or clustering behaviour on rewarming rate and that disturbance by normothermic bats contributes to the overall increase in arousal frequency. We found that arousals of infected bats became more synchronised than those of controls as hibernation progressed but the pattern was not consistent with social thermoregulation. When a bat rewarmed from torpor, it was often followed in sequence by up to seven other bats in an arousal "cascade". Moreover, rewarming rate did not differ between infected and uninfected bats, was not affected by clustering and did not change over time. Our results support our second hypothesis and suggest that disturbance, not social thermoregulation, explains the increased synchronisation of arousals. Negative pathophysiological effects of WNS on energy conservation may therefore be compounded by maladaptive changes in behaviour of the bats, accelerating fat depletion and starvation.

1. Introduction

Pathogenic infections trigger behavioural responses of hosts. These can reduce severity of disease, benefitting the host [1–3], or increase parasite survival or transmission, disadvantaging the host [1,3–5]. For example, a common response of hosts to infection is to increase body

* Corresponding author.
E-mail address: c.willis@uwinnipeg.ca (C.K.R. Willis).
[1] Present address: Department of Animal Ecology and Conservation, Biocentre Grindel, Hamburg University, Hamburg 20146, Germany.

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temperature (T_b) outside the pathogen's optimal thermal zone [6]. In ectothermic animals, this response often involves selection of ambient temperatures (T_a) that are warmer than hosts normally experience [7–10]. On the other hand, selecting a cooler T_a can slow the growth of a parasite and reduce the chance of successful development within the host [11]. However, the relationship between T_a and host/parasite survival is seldom linear [12] and environmental constraints might further limit hosts' responses if a suitable T_a is not available.

Many mammalian and avian species save energy during adverse weather or resource scarcity by using torpor, a controlled physiological state of reduced T_b and metabolism [13]. Torpid animals are usually only capable of slow, poorly co-ordinated movements and must, therefore, select roost or nest sites with suitable microclimates for torpor expression while they are still normothermic. Hibernating mammals use long-term bouts of torpor that can last days to weeks. During these long torpor bouts, T_b is typically thermoconforming and microclimates selected by many hibernators are often highly stable. Immune responses of the few hibernating mammals that have been examined are down-regulated [14,15]. An immune response might occur during normothermia but, for many hibernators, maximising time in torpor is critical for winter survival. The combination of diminished temperature-dependent physiological processes, restricted behavioural movements, extreme energy limitation and a narrow range of T_a could make torpid mammals particularly susceptible hosts for pathogens that can tolerate low T_a.

The cold-adapted fungus *Pseudogymnoascus destructans* (*Pd*, formerly *Geomyces destructans*; [16]) appears to be such a pathogen, and is the first known pathogen that appears to specialise on torpid mammalian hosts. *Pd* causes white-nose syndrome (WNS) [17,18], an infectious disease that has devastated bat populations in eastern North America [19,20]. *Pd* invades exposed skin of torpid bats during hibernation and this infection appears to disrupt regular torpor patterns resulting in the premature exhaustion of fat reserves and starvation [18,21–23]. Bats affected by WNS show a progressive increase in the frequency of periodic arousals from torpor (i.e., decrease in torpor bout duration) compared to control animals [18,23] which could reflect increased fluid and electrolyte loss across damaged wing tissue [24,25]. In addition to these physiological changes, infected bats also display behavioural changes including altered activity levels and reduced clustering [20,26,27]. Overall, *Pd* affects physiological and behavioural aspects of bat hibernation in ways that disrupt the tight winter energy budgets of bats.

Compared to the torpid state, periodic arousals to normothermia consume a disproportionally large fraction of a hibernator's winter fuel supply and account for ~85% of the over-winter energy budget [28]. Many heterotherms decrease arousal costs by passively rewarming from torpor with increasing T_a or solar radiation [29–31] but environmental conditions in hibernacula of WNS-affected bat species are highly stable, eliminating this possibility. Some species such as little brown bats (*Myotis lucifugus*) often roost in large clusters which could help them conserve water and prolong torpor bouts, reduce thermoregulatory energy expenditure during torpor if hibernaculum T_a falls below the lower critical torpid T_b, and/or reduce energy expenditure during arousals if individuals tightly synchronise rewarming and share costs via social thermoregulation [32–36]. There is some evidence for synchronised arousals in *M. lucifugus* and, although their energetic implications are not fully understood, this could reflect social thermoregulation [36]. By passively absorbing heat from adjacent, normothermic individuals during rewarming, individual bats could reduce energy expenditure during arousals and conserve fat reserves [33]. This behaviour could increase the chance of survival for WNS-affected bats if it helps them endure the increased arousal frequency associated with *Pd* infection.

There are other explanations for synchronised arousals in hibernating bats. First, in many hibernators the timing of arousals follows a circadian pattern entrainable to the light–dark cycle (e.g., [37–39]) and synchronised arousals could reflect an active circadian rhythm. Some hibernating bats time periodic arousals with their usual foraging time around sunset [40,41] while, for other species like *M. lucifugus*, that store large fat reserves and hibernate in caves with few external environmental cues, the rhythm can be weakened [42] or absent [36,43–45]. During mid-hibernation, periodic arousals of free-ranging, healthy *M. lucifugus* do not coincide with sunset but occur any time during the day or night and are often at least partially synchronised with cluster-mates [36,42]. This synchronisation could be beneficial if it allows social thermoregulation but could also reflect a detrimental consequence of roosting in large groups. Torpid bats are sensitive to even non-tactile disturbance [46–48] and it is possible that synchronised arousals reflect disturbance by normothermic, active conspecifics [47].

Potential interactions between physiological (i.e., increased arousal frequency) and behavioural changes (i.e., reduced clustering behaviour) that occur with WNS have not been investigated and no data have addressed how WNS may affect temporal patterns of arousal in hibernating bats. Therefore, we investigated arousal timing and rewarming rates in relation to clustering behaviour in *M. lucifugus* inoculated with *Pd*. We tested two competing hypotheses about effects of WNS on the behaviour and physiology of arousal. First, we tested whether infected bats adjust arousal physiology as part of an adaptive response to help compensate for the increased frequency of energetically expensive arousals. This hypothesis leads to three predictions: 1) Infected bats will more tightly synchronise arousals with colony-mates compared to controls and share arousal costs by rewarming simultaneously; 2) rewarming rates during synchronised arousals, or during arousals of clustered bats, will be slower than those during unsynchronised arousals, or arousals of solitary bats, because passive rewarming exploiting an exogenous heat source (e.g., an adjacent bat) occurs at a slower rate than active rewarming [29]; and 3) unsynchronised arousals by infected bats will be characterised by faster rewarming rates than unsynchronised arousals of controls because, in the absence of a passive heat source, faster rewarming is less costly than slow rewarming [49]. Second, we tested the alternative hypothesis that changes in arousal timing for bats with WNS are maladaptive consequences of infection which partially reflect disturbance of torpid bats by infected conspecifics. This hypothesis leads to two predictions: 1) As for the social thermoregulation hypothesis above, a greater proportion of arousals by infected individuals will occur at about the same times compared to those of controls. However, if disturbance rather than social thermoregulation influences arousal behaviour, arousals of infected colony-mates should occur in a sequence or “cascade” rather than simultaneously, with relatively few individuals normothermic at precisely the same time; and 2) neither arousal synchrony nor clustering will influence rewarming rates of infected or control bats.

2. Materials and methods

2.1. Housing

Details on methods and other data from this experiment have been published previously [18,25,27] and so are presented briefly here. The study was carried out at the Western College of Veterinary Medicine, University of Saskatchewan, Canada between November 2010 and March 2011. 54 male *M. lucifugus* were brought into captivity from a WNS-free cave in central Manitoba, Canada and housed in nylon mesh enclosures (Reptarium; Apogee, Dallas, TX, USA) within environment chambers (VWR BOD 2020; VWR International, Mississauga, ON, Canada). Consistent with many natural hibernacula, chambers were maintained at 7 °C and >97% relative humidity and kept in complete darkness without provision of food. Water was available ad libitum.